

## CLAIMS:

1. A process for producing recombinant calf-chymosin which comprises the steps of isolating calf-chymosin gene, cloning the same in bacterial expression vector PET21b, transforming said cloned vector into cells of E.coli, fermenting said E.coli strains to produce pro-chymosin, converting said pro-chymosin to chymosin and subsequently recovering the recombinant calf-chymosin.
2. The process as claimed in claim 1, wherein calf-chymosin gene is obtained by isolating RNA from the fourth stomach of calf tissue, synthesising a first strand of cDNA therefrom by treating the same with a reverse primer such as 5'-TGT GGG GAG AGT GAG GTT CTT GGT C-3' and then with a forward primer such as 5'-ATG AGG TGT CTC GTG GTG CTA CTT 3 and with a reverse primer such as 5'TGT GGT GAC AGT GAG GTT CTT GGT C-3'.
3. The process as claimed in claims 1 and 2 wherein said C DNA is ligated at small site of pBSSK+ plasmid and then transformed into TOP 10 cells of E.coli.
4. The process as claimed in claim 3 wherein said recombinant clones were identified and treated with a forward primer such as 5'-GAT ATA CAT ATG GCT AGC ATC ACT AGG ATC CCT CTG TAC 3' and reverse primer such as 5' GCA GTA AGC TTG ACA GTG TTC CTT GGT CAG CG-3' containing Nde I and Hind III sites to obtain an amplified fragment.
5. The process as claimed in claim 4 wherein said amplified fragment is transformed into cells of E.coli for expressing said chymosin gene.

6. The process as claimed in any of the preceding claims wherein said E.coli cells containing recombinant calf chymosin gene is fermented in a medium containing 12g/L peptone, 24g/L of yeast extract and 10g/L of sodium chloride in the presence of supplements for fermentation and the suspended cells produced on completion of fermentation is lysed, chilled and pH adjusted to 8 before incubating at room temperature and the supernatent containing prochymosin is separated.
7. The process as claimed in claim 6, wherein the pH of said prochymosin containing supernatent is adjusted to 2 at room temperature and further incubated for about 6 hrs with gentle stirring and filtered.
8. The process as claimed in claim 7 wherein the pH of said filtrate is adjusted to about 5 and further incubated, filtered and treated with a solution containing sodium benzoate and thereafter a solution containing and sodium chloride to activate prochymosin to chymosin.
9. The process as claimed in claim 8 wherein the filtrate obtained after the addition of sodium benzoate solution is treated with a solution of sodium chloride under stirring and cooking, and the precipitate suspended in a chilled solution of 0.2M glycine with 0.001M EDTA and thereafter treated with 0.23% solution of sodium benzoate and stored under cooling.
10. The process as claimed in claim 9 wherein said chymosin obtained is formulated with 10% of sodium chloride and 0.2% of Trehalose.

11. Recombinant calf-chymosin having the following amino acid sequence:

MetAlaSerIle ThrArgIle ProLeuTyr LysGlyLysSer LeuArgLys AlaLeuLys  
 1 ATGGCTAGCA TCACTAGGAT CCCTCTGTAC AAAGGCAAGT CTCTGAGGAA GGCGCTGAAG  
   TACCGATCGT AGTGATCCTA GGGAGACATG TTTCCGTTCA GAGACTCCTT CCGCGACTTC  
   GluHisGlyLeu LeuGluAsp PheLeuGln LysGlnGlnTyr GlyIleSer SerLysTyr  
 61 GAGCATGGC TTCTGGAGGA CTTCCGTGCAG AAACAGCAGT ATGGCATTAG CAGCAACTAC  
   CTCGTACCG AAGACCTCCT GAAGGACGTC TTTGTCGTCA TACCGTAGTC GTCGTTCATG  
   SerGlyPheGly GluValAla SerValPro LeuThrAsnTyr LeuAspSer GlnTyrPhe  
 121 TCCGGCTTCG GGGAGGTGGC CAGCGTGCCTC CTGACCAACT ACCTGGATAG TCAGTACTTT  
   AGGCCGAAGC CCCTCCACCG GTCGCACGGG GACTGGTTGA TGGACCTATC AGTCATGAAA  
   GlyLysIleTyr LeuGlyThr ProProGln GluPheThrVal LeuPheAsp ThrGlySer  
 181 GGGAAAGATCT ACCTCGGGAC CCCGCCAG GAGTTCACCG TGCTGTTGA CACTGGCTCC  
   CCCTCTAGA TGGAGCCCTG GGGCGGGGTC CTCAAGTGGC ACGACAAACT GTGACCGAGG  
   SerAspPheTrp ValProSer IleTyrCys LysSerAsnAla CysLysAsn HisGlnArg  
 241 TCTGACTTCT GGGTACCCCTC TATCTACTGC AAGAGCAATG CCTGAAAAA CCACCAGCGC  
   AGACTGAAGA CCCATGGGAG ATAGATGACG TTCTCGTTAC GGACGTTTT GGTGGTCGCG  
   PheAspProArg LysSerSer ThrPheGln AsnLeuGlyLys ProLeuSer IleHisTyr  
 301 TTCGACCCGA GAAAGTCGTC CACCTTCCAG AACCTGGCA AGCCCCTGTC TATCCACTAC  
   AAGCTGGCT CTTTCAGCAG GTGGAAGGTC TTGGACCCGT TCAGGGACAG ATAGGTGATG  
   GlyThrGlyLys MetGlnGly IleLeuGly TyrAspThrVal ThrValSer AsnIleVal  
 361 GGGACAGGCCA AGATGCAGGG GATCCTGGC TATGACACCG TCACTGTCTC CAACATTGTG  
   CCCTGTCCGT TCTACGTCCC CTAGGACCCG ATACTGTGGC AGTGACAGAG GTTGTAAACAC  
   AspIleGlnGln ThrValVal LeuSerThr GlnGluProGly AspValPhe ThrTyrAla  
 421 GACATCCAGC AGACAGTAGT CCTGAGCACC CAGGAGCCCG GGGACGTCTT CACCTATGCC  
   CTGTAGGTCTG TCTGTCTAC GGACTCGTGG GTCCTGGC CCCTGCAGAA GTGGATAACGG  
   GluPheAspGly IleLeuGly MetAlaTyr ProSerLeuAla SerGluVal LeuAspThr  
 481 GAATTGACG GGATCCTGGG GATGGCGTAC CCCTCGCTGG CCTCAGAAAGT ACTCGATACC  
   CTTAAGCTGC CCTAGGACCC CTACCGCATG GGGAGCGACC GGAGTCTTCA TGAGCTATGG  
   GlyPheAspAsn MetMetAsn ArgHisLeu ValAlaGlnAsp ValPheSer ValTyrMet  
 541 GGCTTGACA ACATGATGAA CAGGCACCTG CCGAAACTGT TGTACTACTT GTCCGTGGAC  
   AspArgAsnGly GlnGlyAsn MetPheThr LeuGlyAlaIle AspProSer TyrTyrThr  
 601 GACAGGAATG GGCAGGGAAA CATGTTTAC CTGTCCTTAC CCGTCCCTTT GTACAAATGG  
   GlySerLeuHis TrpValPro ValThrVal CTGGGGCCA TCGACCCGTC CTACTACACA  
 661 GGGTCCCTGC ACTGGGTGCC CGTGACAGTG CCCAGGGACG TGACCCACGG GCACTGTCAC  
   ValThrIleSer GlyValVal ValAlaCys GAGGGTGGCT GTCAGGCCAT CCTGGACACG  
 721 GTCACCATCA GCGGTGTGGT TGTGCCCTGT CAGTGGTAGT CGCCACACCA ACACCGGACA  
   GlyThrSerLys LeuValGly ProSerSer AspIleLeuAsn IleGlnGln AlaIleGly  
 781 GGCACCTCCA AGCTGGTCGG GCCCAGCAGC CCGTGGAGGT TCGACCAGCC CGGGTCGTG  
   AlaThrGlnAsn GlnTyrAsp GluPheAsp IleAspCysAsp AsnLeuSer TyrMetPro  
 841 GCCACACAGA ACCAGTACGA TGAGTTTGAC CGGTGTGTCT TGGTCATGCT ACTCAAACGT  
   ThrValValPhe GluIleAsn GlyLysMet TyrProLeuThr ProSerAla TyrThrSer  
 901 ACTGTGGTCT TTGAGATCAA TGGCAAAATG TGACACCAGA AACTCTAGTT ACCGTTTAC  
   GlnAspGlnGly PheCysThr SerGlyPhe GlnSerGluAsn HisSerGln LysTrpIle

961 CAGGACCAGG GCTTCTGTAC CAGTGGCTTC CAGAGTGAAA ATCATTCCCA GAAATGGATC  
GTCCTGGTCC CGAAGACATG GTCACCGAAG GTCTCACTTT TAGTAAGGGT CTTTACCTAG  
LeuGlyAspVal PheIleArg GluTyrTyr SerValPheAsp ArgAlaAsn AsnLeuVal  
1021 CTGGGGGATG TTTTCATCCG AGAGTATTAC AGCGTCTTG ACAGGGCCAA CAACCTCGTG  
GACCCCCCTAC AAAAGTAGGC TCTCATAATG TCGCAGAAC TGTCCCGGTT GTTGGAGCAC  
GlyLeuAlaLys, AlaIle\*\*\*  
1081 GGGCTGGCCA AAGCCATCTG A  
CCCGACCGGT TTCGGTAGAC T

13. Recombinant calf-chymosin when produced by a process according to any of the preceding claims.